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L5: Entry 1 of 1 File: USPT Nov 6, 2001

US-PAT-NO: 6313172
DOCUMENT-IDENTIFIER: US 6313172 B1

TITLE: Methods and compositions for modulating alpha adrenergic receptor activity

DATE-ISSUED: November 6, 2001

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APPL-NO: 09/ 548410 [PALM]
DATE FILED: April 13, 2000

INT-CL: [07] A61 K 31/17

US-CL-ISSUED: 514/587; 514/580, 514/585
US-CL-CURRENT: 514/587; 514/580, 514/585

FIELD-OF-SEARCH: 514/580, 514/585, 514/587, 568/17, 568/26

PRIOR-ART-DISCLOSED:

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
1 499 485	February 1978	GB	
WO 92/0073	January 1992	WO	

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Medline: 95239789 abs of Journal of neuortrauma by Staub 11(6) pp 679-90, 1994.*
CA:122:105918 abs of WO9404531 by Warshawsky et al, 1994.*
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CA:93:132428 abs of Eur. j. Med. Chem. -Chim. Ther 15(1) by Reiter et al pp 41-53, 1980.*
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Kim et al, "An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat", Pain 50, 355-363 (1992).
Hargreaves et al, "A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia", Pain 32, 77-88 (1988).
Dixon, "Efficient Analysis of Experimental Observations", Ann. Rev. Pharmacol. Toxicol. 20: 441-462 (1980).

ART-UNIT: 161

PRIMARY-EXAMINER: Vollano; Jean F.

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ABSTRACT:

Methods and compositions for the treatment of pain using this area derivatives. Particularly disclosed are new compositions for the treatment of chronic pain, and methods for their use.

10 Claims, 0 Drawing figures
Exemplary Claim Number: 1

BRIEF SUMMARY:

1 BACKGROUND OF THE INVENTION

2 Human adrenergic receptors are integral membrane proteins which have been classified into two broad classes, the alpha and the beta adrenergic receptors. Both types mediate the action of the peripheral sympathetic nervous system upon binding of catecholamines, norepinephrine and epinephrine.

3 Norepinephrine is produced by adrenergic nerve endings, while epinephrine is produced by the adrenal medulla. The binding affinity of adrenergic receptors for these compounds forms one basis of the classification: alpha receptors tend to bind norepinephrine more strongly than epinephrine and much more strongly than the synthetic compound isoproterenol. The preferred binding affinity of these hormones is reversed for the beta receptors. In many tissues, the functional responses, such as smooth muscle contraction, induced by alpha receptor activation are opposed to responses induced by beta receptor binding.

4 Subsequently, the functional distinction between alpha and beta receptors was further highlighted and refined by the pharmacological characterization of these receptors from various animal and tissue sources. As a result, alpha and beta adrenergic receptors were further subdivided into .alpha..sub.1, .alpha..sub.2, .beta..sub.1, and .beta..sub.2 subtypes.

5 Functional differences between .alpha..sub.1 and .alpha..sub.2 receptors have been recognized, and compounds which exhibit selective binding between these two subtypes have been developed. Thus, in WO 92/0073, the selective ability of the R(+) enantiomer of terazosin to selectively bind to adrenergic receptors of the .alpha..sub.1 subtype was reported. The .alpha..sub.1 /.alpha..sub.2 selectivity of this compound was disclosed as being significant because agonist stimulation of the .alpha..sub.2 receptors was said to inhibit secretion of epinephrine and norepinephrine, while antagonism of the .alpha..sub.2 receptor was said to increase secretion of these hormones. Thus, the use of non-selective alpha-adrenergic blockers, such as phenoxybenzamine and phentolamine, was said to be limited by their .alpha..sub.2 adrenergic receptor mediated induction of increased plasma catecholamine concentration and the attendant physiological sequelae (increased heart rate and smooth muscle contraction).

6 For a general background on the .alpha.-adrenergic receptors, the reader's attention is directed to Robert R. Ruffolo, Jr., .alpha.-Adrenoreceptors: Molecular Biology, Biochemistry and Pharmacology, (Progress in Basic and

Clinical Pharmacology series, Karger, 1991), wherein the basis of .alpha..sub.1 /.alpha..sub.2 subclassification, the molecular biology, signal transduction, agonist structure-activity relationships, receptor functions, and therapeutic applications for compounds exhibiting .alpha.-adrenergic receptor affinity was explored.

7 The cloning, sequencing and expression of alpha receptor subtypes from animal tissues has led to the subclassification of the .alpha..sub.1 adrenoreceptors into .alpha..sub.1A, .alpha..sub.1B, and .alpha..sub.1D. Similarly, the .alpha..sub.2 adrenoreceptors have also been classified .alpha..sub.2A, .alpha..sub.2B, and .alpha..sub.2C receptors. Each .alpha..sub.2 receptor subtype appears to exhibit its own pharmacological and tissue specificities. Compounds having a degree of specificity for one or more of these subtypes may be more specific therapeutic agents for a given indication than an .alpha..sub.2 receptor pan-agonist (such as the drug clonidine) or a pan-antagonist.

8 Among other indications, such as the treatment of glaucoma, hypertension, sexual dysfunction, and depression, certain compounds having alpha 2 adrenergic receptor agonist activity are known analgesics. However, many compounds having such activity do not provide the activity and specificity desirable when treating disorders modulated by alpha-2 adrenoreceptors. For example, many compounds found to be effective agents in the treatment of pain are frequently found to have undesirable side effects, such as causing hypotension and sedation at systemically effective doses. There is a need for new drugs that provide relief from pain without causing these undesirable side effects. Additionally, there is a need for agents which display activity against pain, particularly chronic pain, such as chronic neuropathic and visceral pain.

9 British Patent 1 499 485, published February 1, 1978 describes certain thiocarbamide derivatives; some of these are said to be useful in the treatment of conditions such as hypertension, depression or pain.

10 OBJECTS OF THE INVENTION

11 It is an object of the invention to provide compounds and compositions useful in treating disorders modulated by alpha-2 adrenoreceptors.

12 It is an object of this invention to provide novel compounds having substantial analgesic activity in the treatment of chronic pain, regardless of origin. Chronic pain may be, without limitation, visceral, inflammatory or neuropathic in origin. Such chronic pain may arise as a result of, or be attendant to, conditions including without limitation: arthritis, (including rheumatoid arthritis), spondylitis, gouty arthritis, osteoarthritis, juvenile arthritis, and autoimmune diseases including, without limitation, lupus erythematosus.

13 These compositions can also be used within the context of the treatment of chronic gastrointestinal inflammations, Crohn's disease, gastritis, irritable bowel disease (IBD) and ulcerative colitis; and in treatment of visceral pain, including pain caused by cancer or attendant to the treatment of cancer as, for example, by chemotherapy or radiation therapy.

14 These compositions can be used within the context of the treatment of other chronic pain symptoms, and especially in the treatment of chronic forms of neuropathic pain, in particular, without limitation, neuralgia, herpes, deafferentation pain, and diabetic neuropathies.

15 It is also an object of this invention to provide novel compounds for treating ocular disorders, such as ocular hypertension, glaucoma, hyperemia, conjunctivitis and uveitis.

16 It is also an object of this invention to provide novel compounds for treating the pain associated with substance abuse and/or withdrawal.

17 It is a still further object of this invention to provide such compounds which have good activity when delivered by peroral, parenteral, intranasal,

ophthalmic, and/or topical dosing, or injection.

18 It is also an object of this invention to provide methods of treating pain through the therapeutic administration of the compounds disclosed herein.

19 It is further an object of the present invention to provide methods of treating conditions known to be susceptible to treatment through alpha 2 adrenergic receptors.

20 SUMMARY OF THE INVENTION

21 The present invention is directed to compounds having the formula: ##STR1##

22 wherein R.sub.1 is F or H, R.sub.2 is Cl or H, and R.sub.3 is F or H; and wherein if R.sub.1 is F then R.sub.2 and R.sub.3 are both H; and if R.sub.1 is H then R.sub.2 is Cl and R.sub.3 is F, and alkyl esters thereof, and pharmaceutically acceptable salts of these compounds.

23 The invention is also directed to methods of treating pain, particularly chronic pain, through the administration of pharmaceutically effective amounts of compounds of the above structure.

24 Further, the invention is directed to methods of treating glaucoma through the administration of a pharmaceutically effective amount of these compounds.

25 DETAILED DESCRIPTION OF THE INVENTION

26 In one aspect, the present invention is directed to compounds of Formula 1: ##STR2##

27 wherein R.sub.1 is F or H, R.sub.2 is Cl or H, and R.sub.3 is F or H; and wherein if R.sub.1 is F then R.sub.2 and R.sub.3 are both H; and if R.sub.1 is H then R.sub.2 is Cl and R.sub.3 is F, and alkyl esters thereof, and pharmaceutically acceptable salts of these compounds.

28 Preferred compounds corresponding to this structure are the following compound (hereinafter termed Formula 2): ##STR3##

29 and the following compound (hereinafter termed Formula 3): ##STR4##

30 and their alkyl esters, and pharmaceutically acceptable derivatives and/or salts of these compounds.

31 Applicants have discovered that these compounds activate .alpha..sub.2 receptors, particularly .alpha..sub.2B receptors. Additionally, these compounds act as a highly effective analgesic, particularly in chronic pain models, with minimal undesirable side effects, such as sedation and cardiovascular depression, commonly seen with agonists of the .alpha..sub.2 receptors.

32 Such compounds may be administered at pharmaceutically effective dosages. Such dosages are normally the minimum dose necessary to achieve the desired therapeutic effect; in the treatment of chromic pain, this amount would be roughly that necessary to reduce the discomfort caused by the pain to tolerable levels. Generally, such doses will be in the range 1-1000 mg/day; more preferably in the range 10 to 500 mg/day. However, the actual amount of the compound to be administered in any given case will be determined by a physician taking into account the relevant circumstances, such as the severity of the pain, the age and weight of the patient, the patient's general physical condition, the cause of the pain, and the route of administration.

33 The compounds are useful in the treatment of pain in a mammal; particularly a human being. Preferably, the patient will be given the compound orally in any acceptable form, such as a tablet, liquid, capsule, powder and the like. However, other routes may be desirable or necessary, particularly if the patient suffers from nausea. Such other routes may include, without exception,

transdermal, parenteral, subcutaneous, intranasal, intrathecal, intramuscular, intravenous, and intrarectal modes of delivery. Additionally, the formulations may be designed to delay release of the active compound over a given period of time, or to carefully control the amount of drug released at a given time during the course of therapy.

34 Another aspect of the invention is drawn to therapeutic compositions comprising the compounds of Formula 1 and alkyl esters and pharmaceutically acceptable derivatives and/or salts of these compounds and a pharmaceutically acceptable excipient. Such an excipient may be a carrier or a diluent; this is usually mixed with the active compound, or permitted to dilute or enclose the active compound. If a diluent, the carrier may be solid, semi-solid, or liquid material that acts as a excipient or vehicle for the active compound. The formulations may also include wetting agents, emulsifying agents, preserving agents, sweetening agents, and/or flavoring agents. If used as in an ophthalmic or infusion format, the formulation will usually contain one or more salt to influence the osmotic pressure of the formulation.

35 In another aspect, the invention is directed to methods for the treatment of pain, particularly chronic pain, through the administration of a compound of Formula 1, and pharmaceutically acceptable alkyl esters, salts, and derivatives thereof to a mammal in need thereof. As indicated above, the compound will usually be formulated in a form consistent with the desired mode of delivery.

36 It is known that chronic pain (such as pain from cancer, arthritis, and many neuropathic injuries) and acute pain (such as that pain produced by an immediate mechanical stimulus, such as tissue section, pinch, prick, or crush) are distinct neurological phenomena mediated to a large degree either by different nerve fibers and neuroreceptors or by a rearrangement or alteration of the function of these nerves upon chronic stimulation. Sensation of acute pain is transmitted quite quickly, primarily by afferent nerve fibers termed C fibers, which normally have a high threshold for mechanical, thermal, and chemical stimulation. While the mechanisms of chronic pain are not completely understood, acute tissue injury can give rise within minutes or hours after the initial stimulation to secondary symptoms, including a regional reduction in the magnitude of the stimulus necessary to elicit a pain response. This phenomenon, which typically occurs in a region emanating from (but larger than) the site of the original stimulus, is termed hyperalgesia. The secondary response can give rise to profoundly enhanced sensitivity to mechanical or thermal stimulus.

37 The A afferent fibers (A.beta. and A.delta. fibers) can be stimulated at a lower threshold than C fibers, and appear to be involved in the sensation of chronic pain. For example, under normal conditions, low threshold stimulation of these fibers (such as a light brush or tickling) is not painful. However, under certain conditions such as those following nerve injury or in the herpesvirus-mediated condition known as shingles the application of even such a light touch or the brush of clothing can be very painful. This condition is termed allodynia and appears to be mediated at least in part by A.delta. afferent nerves. C fibers may also be involved in the sensation of chronic pain, but if so it appears clear that persistent firing of the neurons over time brings about some sort of change which now results in the sensation of chronic pain.

38 By "acute pain" is meant immediate, usually high threshold, pain brought about by injury such as a cut, crush, burn, or by chemical stimulation such as that experienced upon exposure to capsaicin, the active ingredient in chili peppers.

39 By "chronic pain" is meant pain other than acute pain, such as, without limitation, neuropathic pain, visceral pain (including that brought about by Cron's disease and irritable bowel syndrome (IBS)), and referred pain.

DETAILED DESCRIPTION:

1 EXAMPLES

2 Example 1

3 Synthesis of 1-(3-chloro-2-fluorobenzyl)-3-(2-hydroxyethyl)-thiourea (Formula 2)

4 One molar equivalent of 3-chloro-2-fluoro-benzyl bromide (commercially available from e.g., Lancaster Synthesis, Ltd.) is permitted to react with 2 molar equivalents of potassium isothiocyanate in dimethylformamide (DMF) containing 0.5 molar equivalent of NaI at 90.degree. C. for 5 hours with stirring to yield 3-chloro-2-fluorobenzyl isothiocyanate. The reaction mixture is permitted to cool to room temperature, and the solution is diluted with H20 and extracted with ether. The ether phase containing the product is removed and the reaction mixture extracted twice more with fresh ether. The ether phases are combined and the product is concentrated in a Speed Vac.RTM. vacuum centrifuge (using house vacuum) set in a water bath at about 45.degree. C. When the ether has evaporated, the unpurified 3-chloro-2-fluorobenzyl isothiocyanate is a viscous liquid.

5 3.57 g of this compound is mixed with 3 molar equivalents of ethanolamine in acetonitrile, and a catalytic amount (less than 1%) of DMAP (N-N-dimethyl amino pyridine) is added. The reaction mixture is incubated for 14 hours at room temperature with constant stirring. The resulting solution is then concentrated using the Speed Vac.RTM. vacuum centrifuge in a 60.degree. C.-70.degree. C. water bath.

6 The product, 1-(3-chloro-2-fluorobenzyl)-3-(2-hydroxyethyl)-thiourea, is purified by liquid chromatography using 200-300 mesh silica gel in a glass column. The concentrated reaction solution is applied to the column and the column washed with three column volumes of Solvent A (50% ethyl acetate/50% hexanes). The product is then eluted using 2-3 column volumes of Solvent B (10% methanol/90% ethyl acetate). The eluted product is again concentrated in a Speed Vac.RTM. vacuum centrifuge to remove the solvent. The product is then permitted to stand at room temperature, where is crystallizes spontaneously. The crystals are stored in the freezer at -78.degree. C.

7 The product has the following spectroscopic characteristics: .sup.1 H NMR (D.sub.6 DMSO, 300 MHz) .delta.7.98 (br s, 1 H), 7.63 (br s, 1 H), 7.46 (t, J=3.9 Hz, 1 H), 7.32-7.18 (m, 2 H), 4.78 (br s, 1H), 4.72 (d, J=3.9 Hz, 2 H), 3.47 (br s, 4 H).

8 In order to compare the biological activity of 1-(3-chloro-2-fluorobenzyl)-3-(2-hydroxyethyl)-thiourea with that of the 2-fluorobenzyl derivative (FORMULA 4) and the 4-flurobenzyl derivative (FORMULA 3), FORMULA 4 is synthesized using 2-fluoro-benzyl bromide (also commercially available) as the starting material. FORMULA 3 is synthesized using commercially purchased 4-fluorobenzyl isothiocyanate. Other synthetic steps are analogous to those used above to synthesize the compound of FORMULA 2.

9 The 2-fluorobenzyl isothiourea derivative (hereinafter termed FORMULA 4) has the following formula: ##STR5##

10 The physiological activity of these compounds was tested using four models: a rat locomotor model to assess sedation, an assay of cardiovascular activity in monkeys, a rat thermal paw withdrawal assay (Dirig et al., J. Neurosci. Methods 76:183-191 (1997) to test the alleviation of acute pain, and the rat spinal nerve ligation allodynia model (Kim and Chung, Pain 50:355-363 (1992) to assess the alleviation of neuropathic pain and central sensitization typical of chronic pain. As is known to those of skill in the art, these tests are established pharmacological methods for determining chronic pain, respectively, of pharmaceutical agents.

11 Example 2

12 Sedative Activity

13 To test sedation, six male Sprague-Dawley rats were given up to 3 mg/kg of each compound in a saline or DMSO vehicle by intraperitoneal injection (i.p.). Sedation was graded 30 minutes following administration of the drug by monitoring locomotor skills as follows.

14 The Sprague-Dawley rats are weighed and 1 ml/kg body weight of an appropriate concentration (ie. 3 mg/ml for a final dose of 3 mg/kg) drug solution is injected intraperitoneally. FORMULA 3 is formulated in approximately 10% DMSO and FORMULA 2 and FORMULA 4 are formulated in 50% DMSO. The results are compared to 29 historical controls that were injected with 1 ml/kg saline or 50% DMSO. Rat activity is then determined 30 minutes after injection of the drug solution. Rats are placed in a dark covered chamber and a digicom analyzer (Omnitech Electronic) quantitates their exploratory behavior for a five-minute period. The machine records each time the rat 30 interrupts an array of 32 photoelectric beams in the X and Y orientation.

15 The results show that, in comparison to the appropriate vehicle controls, none of the compounds caused a statistically significant reduction in the exploratory activity of the rats. FORMULA 2 and FORMULA 3 were tested at 1 mg/kg and FORMULA 4 was tested at 3 mg/kg. Thus, the compounds are not sedating.

16 Example 3

17 Effects on Cardiovascular System

18 To test the effect of the compounds on the cardiovascular system, six cynomolgus monkeys were given 500 .mu.g/kg of each compound by intravenous injection (i.v.). The effects of each compound on the animals' blood pressure and heart rate was measured at time intervals from 30 minutes to six hours following administration of the drug. The peak change from a baseline measurement taken 30 minutes before drug administration is recorded using a blood pressure cuff modified for use on monkeys.

19 The monkeys are weighed (approximately 4 kg) and an appropriate volume (0.1 ml/kg) of a 5 mg/ml solution of each compound formulated in 10% DMSO is injected into the cephalic vein in the animals' arms. Cardiovascular measurements are made with a BP 100S automated sphygmomanometer (Nippon Colin, Japan) at 0.5, 1, 2, 4 and 6 hours.

20 The results show that, in comparison to the predrug control, none of the compounds have any detectable effect on the cardiovascular system.

21 Example 4

22 Alleviation of Acute Pain

23 Models to measure sensitivity to acute pain have typically involved the acute application of thermal stimuli; such a stimulus causes a programmed escape mechanism to remove the affected area from the stimulus. The proper stimulus is thought to involve the activation of high threshold thermoreceptors and C fiber dorsal root ganglion neurons that transmit the pain signal to the spinal cord.

24 The escape response may be "wired" to occur solely through spinal neurons, which receive the afferent input from the stimulated nerve receptors and cause the "escape" neuromuscular response, or may be processed supraspinally--that is, at the level of the brain. A commonly used method to measure nociceptive reflexes involves quantification of the withdrawal or licking of the rodent paw following thermal excitation. See Dirig, D. M. et al., J. Neurosci. Methods 76:183-191 (1997) and Hargreaves, K. et al., Pain 32:77-88 (1988), hereby incorporated by reference herein.

25 In a variation of this latter model, male Sprague-Dawley rats were tested by being placed on a commercially available thermal stimulus device constructed as described in Hargreaves et al. This device consists of a box containing a glass

plate. The nociceptive stimulus is provided by a focused projection bulb that is movable, permitting the stimulus to be applied to the heel of one or both hindpaws of the test animal. A timer is actuated with the light source, and the response latency (defined as the time period between application of the stimulus and an abrupt withdrawal of the hindpaw) is registered by use of a photodiode motion sensor array that turns off the timer and light. Stimulus strength can be controlled by current regulation to the light source. Heating is automatically terminated after 20 seconds to prevent tissue damage.

26 Four test animals per group were weighed (approximately 0.3 kg) and injected intraperitonealy (i.p.) with 1 ml/kg of each compound formulated in approximately 50% dimethylsulfoxide (DMSO) vehicle. Animals received a 0.3 mg/kg and a 3 mg/kg dose of the three compounds. Rats were acclimated to the test chamber for about 15 minutes prior to testing. The paw withdrawal latency was measured at 30, 60 and 120 minutes after drug administration. The right and left paws were tested 1 minute apart, and the response latencies for each paw were averaged. Stimulus intensity was sufficient to provide a temperature of 45-50 degrees centigrade to each rat hindpaw.

27 The results show that none of the compounds provide analgesic effects in this bioassay of acute pain. The response latencies for rats treated with the compounds were not statistically different from the response latencies of the rats treated with vehicle alone.

28 Example 5

29 Alleviation of Chronic Pain

30 A model for chronic pain (in particular peripheral neuropathy such as causalgia) involves the surgical ligation of the L5 (and optionally the L6) spinal nerves on one side in experimental animals. Rats recovering from the surgery gain weight and display a level of general activity similar to that of normal rats. However, these rats develop abnormalities of the foot, wherein the hindpaw is moderately exerted and the toes are held together. More importantly, the hindpaw on the side affected by the surgery appears to become sensitive to pain from low-threshold mechanical stimuli, such as that producing a faint sensation of touch in a human, within about 1 week following surgery. This sensitivity to normally non-painful touch is called "tactile allodynia" and lasts for at least two months. The response includes lifting the affected hindpaw to escape from the stimulus, licking the paw and holding it in the air for many seconds. None of these responses is normally seen in the control group.

31 Rats are anesthetized before surgery. The surgical site is shaved and prepared either with betadine or Novacaine. Incision is made from the thoracic vertebra XIII down toward the sacrum. Muscle tissue is separated from the spinal vertebra (left side) at the L4-S2 levels. The L6 vertebra is located and the transverse process is carefully removed with a small rongeur to expose the L4-L6 spinal nerves. The L5 and L6 spinal nerves are isolated and tightly ligated with 6-0 silk thread. The same procedure is done on the right side as a control, except no ligation of the spinal nerves is performed.

32 A complete hemostasis is confirmed, then the wounds are sutured. A small amount of antibiotic ointment is applied to the incised area, and the rat is transferred to the recovery plastic cage under a regulated heat-temperature lamp. On the day of the experiment, at least seven days after the surgery, six rats per test group are administered the test drugs by intraperitoneal (i.p.) injection or oral gavage. For i.p. injection, the compounds are formulated in approximately 50% DMSO and given in a volume of 1 ml/kg body weight. FORMULA 2 was tested at doses ranging between 1 and 300 .mu.g/kg, FORMULA 3 was tested at doses between 0.1 and 3 mg/kg and FORMULA 4 was tested at doses of 0.3 and 3 mg/kg. FORMULA 2 was also administered by oral gavage at doses of 0.1, 0.3 and 1 mg/kg body weight to 24-hour fasted rats. A volume equal to 1 ml/kg body weight of an appropriate concentration (ie. 1 mg/ml for a 1 mg/kg dose) of FORMULA 2 formulated in approximately 50% DMSO was injected using an 18-gauge, 3-inch gavage needle that is slowly inserted through the esophagus into the stomach.

33 Tactile allodynia is measured prior to and 30 minutes after drug administration using von Frey hairs that are a series of fine hairs with incremental differences in stiffness. Rats are placed in a plastic cage with a wire mesh bottom and allowed to acclimate for approximately 30 minutes. The von Frey hairs are applied perpendicularly through the mesh to the mid-plantar region of the rats' hindpaw with sufficient force to cause slight buckling and held for 6-8 seconds. The applied force has been calculated to range from 0.41 to 15.1 grams. If the paw is sharply withdrawn, it is considered a positive response. A normal animal will not respond to stimuli in this range, but a surgically ligated paw will be withdrawn in response to a 1-2 gram hair. The 50% paw withdrawal threshold is determined using the method of Dixon, W. J., Ann. Rev. Pharmacol. Toxicol. 20:441-462 (1980). The post-drug threshold is compared to the pre-drug threshold and the percent reversal of tactile sensitivity is calculated based on a normal threshold of 15.1 grams.

34 The results showed that FORMULA 4 had no analgesic activity at doses up to 3 mg/kg. Surprisingly, AGN 196204 and FORMULA 2 were both able to reduce the response to the tactile stimuli that indicate tactile allodynia. FORMULA 3 reversed the allodynic pain by 34% at an i.p. dose of 0.3 mg/kg, 32% at 1 mg/kg and 26% at 3 mg/kg. FORMULA 2 reversed the allodynia by 55% at an i.p. dose of 3 .mu.g/kg, 85% at 10 .mu.g/kg, 90% at 30 .mu.g/kg, 95% at 100 .mu.g/kg and 92% at 300 .mu.g/kg. The oral doses of FORMULA 2 ranging from 0.1 to 1 mg/kg alleviated the allodynic pain by 82-86%. Thus, FORMULA 3 and FORMULA 2 are analgesic in a model of chronic pain.

35 Example 6

36 Treatment of Allodynia with FORMULA 3

37 A 50 year old male in generally good physical condition suffers from serious pain to his upper body due caused by contact of his skin with his clothing. The patient is unable to wear clothing on his upper body without severe pain. His symptoms suggest a diagnosis of shingles.

38 The patient is given a therapeutically effective oral dose of FORMULA 3 in capsule form as needed for the treatment of pain. Following two day's treatment, the patient reports that the allodynia resulting from shingles is markedly reduced, and that he is able to wear clothing on his upper body with greater comfort.

39 Example 6

40 Treatment of Allodynia with FORMULA 2

41 Same facts as in Example 5, except the patient is given a therapeutically effective oral dose of FORMULA 2 in capsule form as needed for the treatment of pain. Following two day's treatment, the patient reports that the allodynia resulting from shingles is markedly reduced, and that he is able to wear clothing on his upper body with greater comfort.

42 Example 7

43 Treatment of Visceral Pain with FORMULA 3

44 A 43 year old female patient suffering from colon cancer and undergoing chemotherapy experiences severe visceral pain associated with this primary condition. Treatment of this pain with opiates have been ineffective to provide substantial relief.

45 The patient is given a therapeutic amount of FORMULA 3 by intravenous infusion in a pharmaceutically acceptable vehicle. The treatment is given twice daily. After two days the patient reports a significant alleviation in the visceral pain associated with her condition.

Example 8

Treatment of Visceral Pain with FORMULA 2

Under the same facts as Example 7, except the patient is given FORMULA 2 instead of FORMULA 3. After two days the patient reports a significant alleviation in the visceral pain associated with her condition.

The examples contained herein are intended to be exemplary only, and do not limit the scope of the invention, which is defined by the claims that conclude this specification.

CLAIMS:

We claim:

1. A method of treating pain in a mammal in need thereof comprising the step: administering to said patient a therapeutically effective dose of a composition comprising a compound represented by the formula: ##STR6##

wherein R.sub.1 is F or H, R.sub.2 is Cl or H, and R.sub.3 is F or H; and wherein if R.sub.1 is F then R.sub.2 and R.sub.3 are both H; and if R.sub.1 is H then R.sub.2 is Cl and R.sub.3 is F, and pharmaceutically acceptable salts of these compounds.

2. The method of claim 1 wherein said compound is represented by the formula ##STR7##

and alkyl esters thereof, and pharmaceutically acceptable salts of these compounds.

3. The method of claim 2 wherein said compound is administered orally.

4. The method of claim 1 wherein said compound is represented by the formula ##STR8##

and pharmaceutically acceptable salts of these compounds.

5. The method of claim 4 wherein said compound is administered orally.

6. The method of claim 1 wherein said pain is chronic pain.

7. The method of claim 1 wherein said pain is neuropathic pain.

8. The method of claim 1 wherein said pain is visceral pain.

9. The method of claim 1 wherein said pain is referred pain.

10. The method of claim 1 wherein said pain is allodynic pain.

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L6: Entry 1 of 1 File: USPT Jan 5, 1999

US-PAT-NO: 5856329
DOCUMENT-IDENTIFIER: US 5856329 A

TITLE: Method of using (2-imidazolin-2-ylamino) quinoxalines in treating ocular neural injury

DATE-ISSUED: January 5, 1999

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ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
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APPL-NO: 08/ 496262 [PALM]
DATE FILED: June 28, 1995

INT-CL: [06] A61 K 31/495

US-CL-ISSUED: 514/255; 514/912
US-CL-CURRENT: 514/249; 514/912

FIELD-OF-SEARCH: 514/255, 514/912

PRIOR-ART-DISCLOSED:

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Search Selected | Search ALL

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PCT/US95/13624	October 1995	WO	

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Janet B. Serle, "Pharmacological Advances in the Treatment of Glaucoma", Drugs Aging, 5 (3), Sep. 1994, 156-170.
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Stys et al, "Compound action potential of nerve recorded by suction electrode: a theoretical and experimental analysis", Brain Research, 546 (1991) pp. 18-32.
Burke et al, "Ocular effects of a relatively selective .alpha.2 agonist (UK-14, 304-18) in cats, rabbits and monkeys", Current Eye Research, vol. 5, No. 9, 1986, pp. 665-676.
Michel et al, "Keeping an eye on the I site: imidazoline-preferring receptors", TiPS--Oct. 1992 [vol. 13], pp. 369-370.
Chemical Abstracts 122: 96456 (1994). Gablet et al.

ART-UNIT: 164

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ABSTRACT:

A method according to which neuroprotection is conferred upon ocular nerve cells by administration of a drug of formula I to the optic nerve and/or retina of a mammal within a period prior to or following an insult to ocular nerve cells but prior to cell death ##STR1## wherein the 2-imidazolin-2-ylamino group may be in either the 5- or 6-position of the quinoxaline nucleus; x, y and z may be in any of the remaining 5-, 6-, 7- or 8-positions and are selected from hydrogen, halogen, lower alkyl, lower alkoxy or trifluoromethyl; and R is an optional substitutent in either the 2- or 3-position of the quinoxaline nucleus and may be hydrogen, lower alkyl or lower alkoxy is disclosed.

15 Claims, 4 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 2

BRIEF SUMMARY:

1 BACKGROUND OF THE INVENTION

2 The present invention relates to methods for the protection of the optic nerve and the retina of mammalian eyes from noxious provocations including damage by compressive (mechanical) effects of elevated intraocular pressure caused by glaucoma or other etiologic factors and impaired blood flow to these nerves.

3 Glaucoma is a disease of the eye characterized by increased intraocular

pressure. On the basis of its etiology, glaucoma has been classified as primary or secondary. Further, primary glaucoma in adults may be either chronic open-angle or chronic angle-closure. Secondary glaucoma results from pre-existing ocular diseases such as uveitis, intraocular tumor or enlarged cataract.

4 The underlying causes of primary glaucoma are not yet well known. Increased intraocular pressure is due to obstruction or aqueous humor outflow. In chronic open-angle glaucoma, the anterior chamber and its anatomic structures appear normal, but drainage of the aqueous humor is impeded. In acute and chronic angle-closure glaucoma, the anterior chamber is shallow, the filtration angle is narrowed and the iris may obstruct the trabecular meshwork at the entrance to the canal of Schlemm. Dilation of the pupil may push the root of the iris forward against the angle or may produce pupillary block and thus precipitate an acute attack of elevated intraocular pressure. Eyes with narrow anterior chamber angles are predisposed to acute angle-closure glaucoma attacks of varying degrees of severity.

5 Secondary glaucoma is caused by any interference with the flow of aqueous humor from the posterior chamber into the anterior chamber and, subsequently, into the canal of Schlemm. Inflammatory disease of the anterior segment may prevent aqueous escape by causing complete posterior synechia in iris bombe, and may plug the drainage channel with exudates. Other common causes are intraocular tumors, enlarged cataracts, ventral retinal vein occlusion, trauma to the eye, operative procedures and intraocular hemorrhage.

6 Considering all types together, glaucoma occurs in about 2% of all persons over the age of 40 and may be asymptomatic for years before progressing to rapid loss of vision. In cases where surgery is not indicated, topical beta-adrenoceptor antagonists have been the drugs of choice for treating glaucoma. However, alpha adrenergic agonists are awaiting approval for use in the treatment of elevated intraocular pressure and will probably become mainstays in the treatment of this disease once they become available.

7 Various quinoxaline derivatives having alpha.sub.2 agonist activity have been suggested as therapeutic agents by, for example, Danielewicz, et al. in U.S. Pat. Nos. 3,890,319 and 4,029,792. They disclose compounds as regulators of the cardiovascular system which have the following formula: ##STR2## where the 2-imidazolin-2-ylamino group may be in any of the 5-, 6-, 7- or 8-position of the quinoxaline nucleus; x, y and z may be in any of the remaining 5-, 6-, 7- or 8-positions and may be selected from hydrogen, halogen, lower alkyl, lower alkoxy or trifluoromethyl; and R is an optional substituent in either the 2- or 3-position of the quinoxaline nucleus and may be hydrogen, lower alkyl or lower alkoxy. The presently useful compounds may be prepared in accordance with the procedures outlined by Danielewicz, et al. The contents of both U.S. Pat. Nos. 3,890,319 and 4,029,792 are hereby incorporated by reference in their entirety.

8 In "Ocular Effects of a Relatively Selective Alpha-2 Agonist (UK-14,304-18) in Cats, Rabbits and Monkeys" [J. A. Burke, et al., Current Eye Rsrch., 5, (9), pp. 665-676 (1986)] the quinoxaline derivative was shown to be effective in reducing intraocular pressure in rabbits, cats and monkeys. Compounds in this study were administered topically to the eye of the study animals. ##STR3## It has long been known that one of the sequelae of glaucoma is damage to the optic nerve head. This damage, referred to as "cupping", results in depressions in areas of the nerve fiber of the optic disk. Loss of sight from this cupping is progressive and can lead to blindness if the condition is not treated effectively.

9 Unfortunately lowering intraocular pressure by administration of drugs or by surgery to facilitate outflow of the aqueous humor is not always effective in obviating damage to the nerves in glaucomatous conditions. This apparent contradiction is addressed by Cioffi and Van Buskirk [Surv. of Ophthalmol., 38, Suppl. p. S107-16, discussion S116-17, May 1994] in the article, "Microvasculature of the Anterior Optic Nerve". The abstract states:

10 The traditional definition of glaucoma as a disorder of increased intraocular pressure (IOP) oversimplifies the clinical situation. Some glaucoma patients never have higher than normal IOP and others continue to develop optic nerve damage despite maximal lowering of IOP. Another possible factor in the etiology of glaucoma may be regulation of the regional microvasculature of the anterior optic nerve. One reason to believe that microvascular factors are important is that many microvascular diseases are associated with glaucomatous optic neuropathy.

11 Subsequent to Cioffi, et al., Matusi published a paper on the "Ophthalmologic aspects of Systemic Vasculitis" [Nippon Rinsho, 52 (8), p. 2158-63, August 1994] and added further support to the assertion that many microvascular diseases are associated with glaucomatous optic neuropathy. The summary states:

12 Ocular findings of systemic vasculitis, such as polyarteritis nodosa, giant cell angitis and aortitis syndrome were reviewed. Systemic lupus erythematosus is not categorized as systemic vasculitis, however its ocular findings are microangiopathic. Therefore, review of its ocular findings was included in this paper. The most common fundus finding in these diseases is ischemic optic neuropathy or retinal vascular occlusions. Therefore several points in diagnosis or pathogenesis of optic neuropathy and retinal and choroidal vaso-occlusion were discussed. Choroidal ischemia has come to be able to be diagnosed clinically, since fluorescein angiography was applied in these lesions. When choroidal arteries are occluded, overlying retinal pigment epithelium is damaged. This causes disruption of barrier function of the epithelium and allows fluid from choroidal vasculatures to pass into subsensory retinal spaces. This is a pathogenesis of serous detachment of the retina. The retinal arterial occlusion formed non-perfused retina. Such hypoxic retina released angiogenesis factors which stimulate retinal and iris neovascularizations and iris neovascularizations may cause neovascular glaucoma.

13 B. Schwartz, in "Circulatory Defects of the Optic Disk and Retina in Ocular Hypertension and High Pressure Open-Angle Glaucoma" [Surv. Ophthalmol., 38, Suppl. pp. S23-24, May 1994] discusses the measurement of progressive defects in the optic nerve and retina associated with the progression of glaucoma. He states:

14 Fluorescein defects are significantly correlated with visual field loss and retinal nerve fiber layer loss. The second circulatory defect is a decrease of flow of fluorescein in the retinal vessels, especially the retinal veins, so that the greater the age, diastolic blood pressure, ocular pressure and visual field loss, the less the flow. Both the optic disk and retinal circulation defects occur in untreated ocular hypertensive eyes. These observations indicate that circulatory defects in the optic disk and retina occur in ocular hypertension and open-angle glaucoma and increase with the progression of the disease.

15 Thus it is evident that there is an unmet need for agents that have neuroprotective effects in the eye that can stop or retard the progressive damage that occurs to the nerves as a result of glaucoma or other ocular afflictions.

16 SUMMARY OF THE INVENTION

17 A new method of protecting the optic nerve and retina of the mammalian eye from damage by glaucoma and other noxious provocations has been discovered. This method comprises administering to the mammal either systemically or by intrabulbar injection an effective amount of one or more of certain aryl-imino-2-imidazolidines (as defined herein), salts thereof and mixtures thereof. This new method is particularly effective when administered as a prophylactic treatment, i.e. before damage to the nerve takes place, or before long-term progression of the disease, such as glaucoma, has taken place.

18 DETAILED DESCRIPTION OF THE INVENTION

19 The drawings will first be briefly described.

DRAWING DESCRIPTION:

DRAWINGS

FIG. 1 is a bar graph showing the percentage of cells killed by treatment with glutamate plotted by number of days since glutamate treatment. A control which was not treated with glutamate has been included to determine cell death which occurred without any such treatment. Also shown are measurements taken after treatment with both AGN191103 and glutamate, and treatment with MK-801 and glutamate. MK-801 is a well known neuroprotective agent in the art. The numbers beneath the bars for glutamate; AGN191103+glutamate; and MK-801+glutamate show the concentrations of glutamate and drug used in each case. At day 8, AGN 191103 and MK-801 show comparable effects in protecting cells from glutamate induced neurotoxicity. Experimental procedures followed in generating the data for this figure are detailed in Example 1.

FIG. 2 shows plots of compound action potentials (CAP) measured for optic nerve fibers: in the left-hand frame, measured at 2 weeks post injury (i.e. after nerve crush) for optic nerve treated with AGN 191103 (the upper line) and for an untreated nerve used as a control (lower line); and in the right-hand frame a comparison CAP of non-injured optic nerve. The scales of the plots are given for each of the frames. The post-injury abscissa scale is 25.times. the scale of the non-injured plot. (Units: millivolts and milliseconds). The value of the compound action potential is calculated as the integral of the area under each curve. The irregularity of the curve is a feature of the dispersion of the compound response; some nerve cells conduct more rapidly than others and so amplitude of the measured voltage varies with time.

FIG. 3 is a bar graph showing the maximal CAP amplitude in microvolts (.mu.V) for cells injured by a optic nerve crush in rats and treated with: 1) vehicle alone; 2) clonidine and 3) AGN191103. Each of the drugs was tested at four different concentrations (administered as a multiple of body weight for the test subject) and is represented by a bar on the chart. Clonidine was chosen as a benchmark .alpha..sub.2 agonist compound with very well defined pharmacology to compare against the test compound AGN 191103. While clonidine did show some neuroprotective activity over vehicle alone, it showed about half the maximal CAP response of AGN191013.

FIG. 4 is a graphic plot of the Visual Evoked Potential Response and shows the electrical potential activity evoked at the surface of the visual cortex (comparable to an electroencephalogram) as a result of visual (light) stimulus. The test is performed in live rats and is a measure of the integrity of the whole visual system from the retina through the optic nerve into the lateral geniculate nucleus and ultimately to the visual cortex located in the back of the brain. The left-hand frame shows the response without nerve crush injury and the right-hand frame shows the responses measured at 2 weeks post-injury for rats treated with AGN191103 above (labeled positive) and control rats below (labeled negative) prior to nerve crush. The scale in .mu.V vs. milliseconds for both plots is shown below the ordinate axis.

DETAILED DESCRIPTION:

1 For a discussion and bibliography regarding the nerve crush model and its significance in evaluating nerve damage and recovery see: "Functional Recovery and Morphological Changes after Injury to the Optic Nerve", Sabel, B. A. and Aschoff, A., Neuropsychobiology, 28, pp. 62-65 (1993).

2 Injury to the mammalian optic nerve, as in any other parts of the mammalian central nervous system (CNS), leads to axonal degeneration followed by a loss of cell bodies, with failure of axonal regrowth from the surviving neurons. Initially, degeneration of the injured nerve is probably attributable to direct neuronal damage. However, the associated physiological and biochemical events

occurring in the nerve immediately after injury are probably responsible for the subsequent progressive degeneration, not only of the directly injured axons, but also of those that escaped the primary damage and largely determine the long-term functional outcome.

3 The immediate injury-induced response strongly influences the subsequent degenerative response. Treatment that reduces or attenuates the immediate response is therefore likely to achieve optimal prevention or delay of the secondary degenerative processes. For monitoring of the immediate response, it is obviously preferable to employ a noninvasive technique. An adaptation of the nicotinamide adenine dinucleotide (NADH) monitoring technique to enable measurement of the earliest post-traumatic events has proved to be a valuable non-invasive approach. Use of the technique allows the immediate effect of the injury to be evaluated in real time and on-line before and after a well-controlled crush injury is inflicted on the adult rat optic nerve in vivo. In this experimental paradigm, measurement of the metabolic activity of the injured optic nerves represent the activity of both injured axons and their associated non neuronal cells, and thus evaluate the potential ability to cope with injurious stresses. The model is also useful for monitoring the activity of various agents that may overcome or mitigate nerve cell damage or death from such stresses.

4 The earliest injury-induced response is a decrease in the energy state of the nerve, under conditions where ischemic events can be completely ruled out. The reduction in the energy state may be related to: 1) postinjury elevation in free fatty acid levels, which may interfere with mitochondrial function and result in uncoupling of electron transport; and 2) a marked rise in intracellular free Ca.sup.2+. It is known that axonal injury is generally followed by an increase in extracellular potassium ions, which stimulate the uptake of Ca.sup.2+ via either voltage sensitive channels (L, T or N type) or receptor-operated Ca.sup.2+ channels. A marked rise in intracellular free Ca.sup.2+ can accelerate processes that are inimical to cell survival, including those involving Ca.sup.2 -dependent enzymes, mainly lipases, proteases and endonucleases, that may cause mitochondrial damage and lead eventually to cell death. The cell, in order to overcome these events, needs more energy to actively restore ionic homeostasis. The combination of increased energy demands and decreased energy conservation resulting from mitochondrial dysfunction at the site of injury may be the major reason for the subsequent irreversible nerve damage and nerve degeneration following injury. Early measurement of metabolic activity could therefore indicate the fate of the axon, its associated glial cells and its non-neuronal cell bodies. It follows from the above that restoration of the mitochondrial activity may be critical in preventing the degenerative process occurring in the nerve after injury.

5 Since the injury inflicted on the nerve in the nerve crush model is a well-controlled, calibrated and reproducible lesion, it is possible to correlate early post-traumatic metabolic deficits and possible mitigation of these by drug or other treatments with long-term morphological and physiological effects.

6 From the foregoing figures and discussion it is apparent that neuroprotection is conferred on nerve cells to both glutamate-induced toxicity and physical insult in the nerve crush model.

7 It has now been discovered that neuroprotection is conferred upon ocular nerve cells by administration of a drug of formula I to the optic nerve and/or retina of a mammal within a period prior to or following an insult to ocular nerve cells but prior to cell death ##STR4## wherein the 2-imidazolin-2-ylamino group may be in either the 5- or 6-position of the quinoxaline nucleus; x, y and z may be in any of the remaining 5-, 6-, 7- or 8-positions and are selected from hydrogen, halogen, lower alkyl, lower alkoxy or trifluoromethyl; and R is an optional substituent in either the 2- or 3-position of the quinoxaline nucleus and may be hydrogen, lower alkyl or lower alkoxy.

8 Definitions

9 The compound identified as AGN 191103 has the chemical structure as ##STR5## shown. It is also known by the chemical nomenclature 6-methyl-(2-imidazolin-2-ylamino) quinoxaline.

10 The neuroprotective agent identified as MK-801 is also known by the name dizocilpine and has the following chemical structure: ##STR6## It is additionally identified and described in the 11th edition of the Merck Index at monograph number 3392.

11 Human Dosage and Administration

12 The methods of this invention are useful in treating any mammal, including humans.

13 According to this invention, mammals are treated with pharmaceutically effective amount of a neuroprotective agent for a period of time and at a time such that noxious provocations to the optic nerve and retina do not kill or permanently damage the nerve cells. Protective agents may be administered orally or by any other appropriate means of delivery described below or known in the art.

14 In accordance with this invention, pharmaceutically effective amounts of a protective agent can be administered alone to treat nerve injury or to prevent nerve cell death. Alternatively a protective agent may be administered sequentially or concurrently with an antiglaucoma drug, e.g. a beta-blocker, an alpha.sub.2 agonist, a muscarinic agent such as pilocarpine, a carbonic anhydrase inhibitor (CAI), or another drug useful in maintaining intraocular pressure (IOP) at normal levels or in lowering elevated IOP. The most effective mode of administration and dosage regimen of protective agent will depend on the type of disease to be treated, the severity and course of that disease, previous therapy, the patient's health status, and response to the drug and the judgment of the treating physician. Generally, the neuroprotective agent should be administered in a dose to achieve a serum or intravitreal concentration of 0.01 nM to 50 nM. Preferably the neuroprotective agent is administered prior to injury to the nerve, but can be administered injury has occurred with lessened effect.

15 Conventional modes of administration and standard dosage regimens of protective agents, e.g. MK-801, can be used. Optimal dosages for coadministration of a drug, e.g. an IOP-lowering drug, with a neuroprotective agent can be determined using methods known in the art. Dosages of neuroprotective agents may be adjusted to the individual patient based on the dosage of the drug with which the agent is coadministered and the response of the patient to the treatment regimen. The protective agent may be administered to the patient at one time or over a series of treatments.

16 An agent that cannot pass the blood/brain barrier, e.g. MK-801, may be administered locally, e.g. intravitreally by intrabulbar injection, or intrathecally. Agents which are capable of crossing the blood/brain barrier, e.g. AGN191103 can be administered systemically, e.g., orally, or intravenously, or by injection.

17 The composition used in these therapies may also be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as tablets, pills, powders, liquid solution or suspension, liposomes, suppositories, injectable and infusible solutions. The compositions also preferably include conventional pharmaceutically acceptable carriers which are known those of skill in the art.

18 The following non-limiting examples describe assays and measurements used in 1) determining protection of nerve cells from glutamate induced toxicity and 2) methods of determining neural protection conferred by neuroprotective agents in a nerve crush model of mechanical injury.

19 EXAMPLE 1

20 Experimental Procedure for Measuring Neural Protection in a Model of Glutamate Induced Excitotoxic Effects on Nerve Cells

21 Low-density rat hippocampal neuronal cultures were prepared by the procedure of Goslin and Banker. Coverslips were cleaned and sterilized in porcelain racks in such a way that they did not stick to one another (Cohen cover glass staining racks, Thomas Scientific). Coverslips (13 mm) were placed in staining racks, rinsed in distilled water (four rinses, 1 min. each) to remove dust and transferred to concentrated HNO.sub.3 for 36 hours. Coverslips were rinsed in distilled water (four changes over 3 hours) and sterilized with dry heat (overnight at 225.degree. C.). The coverslips were transferred to 24-well dishes, one coverslip per well. To support the coverslips above the glia during coculturing, paraffin dots were placed on dishes, and UV irradiation (30 min.) was applied before the coverslips were introduced. One mg/mL of poly-L-lysine hydrobromide (PLL) (Sigma) (MW 30,000-70,000) was dissolved in borate buffer (0.1M, pH 8.5), filtered, sterilized and used to cover each coverslip overnight. The PLL was removed, coverslips were rinsed in distilled water (two washes, 2 hrs. each), plating medium [Eagle's MEM with Earle's salts containing extra glucose (600 mg/L) and 10% horse serum] was added and the dishes were stored in an incubator. Astroglial cultures were prepared from the brains of neonatal rats by a method similar to that described by Levinson and Mc Carthy, except that they were plated at a lower density so that they contained predominantly type 1 astroglia. 10.sup.5 cells were plated in each well. Glial cultures were red with plating medium twice a week and were used after reaching confluence, about 2 weeks after plating. One day before use, the plating medium was removed, neuronal maintenance medium (MEM containing N2 supplements) was added, and incubation continued. 3.times.104 of viable rat hippocampal nerves (E18 embryos) were plated on the PLL-treated coverslips kept in plating medium. After 3-4 hrs, when most of the neurons were attached, the coverslips were transferred to the dishes containing the glial cell in maintenance medium in such a way that the neuronal side was facing the glia, which support neuronal survival and development. To reduce glial proliferation, cytosine arabinoside (1-b-D-arabinofuranosylcytosine)(Calbiochem)(5.times.10M final concentration) was added to the cultures 2 days after plating. At day 6 in culture, cells were treated with 1 mM glutamine or with glutamate together with either AGN-191103--0.1 nM (MW=200) or MK-801--10 nM (2-3 coverslips were used to each treatment).

22 After 24 hrs. of incubation, cells were stained with trypan blue. Live and dead neurons were counted from randomly selected culture fields (5 fields from each coverslip). Percentage of dead cells was calculated.

23 EXAMPLE 2

24 Procedure for Nerve Crush Injury and Measurements of Compound Action Potentials (CAP) Subsequent to Injury

25 Part A

26 Metabolic Measurements

27 Animal utilization was according to the ARVO Resolution on the use of animals in research. Male Sprague-Dawley (SPD) rats weighing 300-400 g were anesthetized with sodium pentobarbitone (intraperitoneally, 35 mg/kg). A cannula was introduced into the trachea for artificial ventilation when required. With the animal's head held in place by a head holder, a lateral canthotomy was performed under a binocular operating microscope and the conjunctiva was incised lateral to the cornea. After separation of the retractor bulbi muscles, the optic nerve was identified and a length of 3 0 3.5 mm was exposed near the eyeball by blunt dissection. The dura was left intact and care was taken not to injure the nerve. The first part of a light guide holder (see below) was inserted under the optic nerve and the nerve was gently eased into the light guide canal. The second part was then fixed in place in such a way that the light guide was located on the surface of the optic nerve 1 mm from the site at which the injury was to be administered.

28 Surface Fluorometry-Reflectometry

29 Monitoring of the intramitonchodrial NADH redox state was based on fluorescence of NADH at 366 nm, resulting in the emission of blue light with a peak intensity at 450 nm, which is unlike its oxidized form, NAD+, which lacks this fluorescence. The source of the 366 nm excitation is a 100-W air-cooled mercury lamp equipped with a strong 366-nm filter (Corning 5860 (7-37) plus 9782 (4-96)). A flexible Y-shaped bundle of optic fibers (light guide) is used to transmit the light to and from the optic nerve, thus making in vivo measurements technically feasible. Excitation light is transmitted through the bundle of excitation fibers to the nerve. The light emitted from the nerve, after being transmitted through a second bundle of fibers, is split in a ration of 90:10 for measurement of the fluorescent light (90%) at 450 nm and the reflected light (10%) at 366 nm by two photomultipliers connected to a one-channel direct current fluorometer-reflectometer. In order to minimize variations among animals, standard signal calibration procedures were applied at the start of the recordings.

30 Changes in the fluorescence and reflectance signals during the experiment are calculated relative to the calibrated signals. This type of calibration, although not absolute, has nevertheless been found to yield reliable and reproducible results from various animals and among different laboratories.

31 Changes in reflected light were correlated with changes in tissue absorption caused by hemodynamic effects and movements of the optic nerve secondary to alteration in arterial blood pressure and nerve volume. The fluorescence measurements are found to be adequately corrected for NADH redox state measurements by subtraction of the reflected light (366 nm) from the fluorescent light (1:1 ratio) to obtain the corrected fluorescence signal.

32 Metabolic Measurements

33 Animals which were still anesthetized were allowed to recover for 30 min. from the surgical procedures described above and were then exposed to anoxic and hyperoxic conditions. An anoxic state was achieved by having the rat breathe in an atmosphere of 100% nitrogen for 2 min., after which it was returned to air. Whenever animals did not return spontaneously to normal breathing, they were ventilated by blowing twice into the trachea. A hyperoxic state was induced by having the animal breathe 100% oxygen for 6-10 min. In order to evaluate the metabolic activity of the optic nerve, the relative changes in reflected and fluorescent light intensities in response to anoxia and to hyperoxia were measured before and after crush injury.

34 Experimental Protocol For Metabolic Measurements

35 Using calibrated cross-action forceps, a well-calibrated moderate crush injury was inflicted to the nerve between the eye and the light guide holder at a pressure corresponding to 120 g for 30 sec.

36 Part B

37 Physiological Measurements

38 Experimental setup for recording compound action potential (CAP): Prior to removal of optic nerves for electrophysiological measurement, the rats were deeply anesthetized with 70 mg/kg pentobarbitone. The skin was removed from the skull and the optic nerves were detached from the eyeballs. Subtotal decapitation was performed and the skull was opened with a rongeur. The cerebrum was displaced laterally, exposing the intracranial portion of the optic nerve. Dissection at the level of the chiasm enabled removal of the whole length of the nerve, which was transferred to vials containing fresh, cold Krebs solution, consisting of: NaCl (125 mM), KCl (5 mM), KH.sub.2 PO.sub.4 (1.2 mM), NaHCO.sub.3 (26 mM), MgSO.sub.4 (0.6 mM), CaCl.sub.2 (24 mM), D-glucose (11 mM), aerated with 95% O.sub.2 and 5% CO.sub.2. The nerves were kept in this solution,

in which electrical activity remained stable for at least 3-4 h. After 1 h of recovery, nerves were immersed in Krebs solution at 37.degree. C. Electrophysiological recording were obtained from the nerve distal to the crush lesion, since the nerves were too small to allow measurement on both sides of the crush. The nerve ends were then connected to two suction Ag--AgCl electrodes immersed in the bathing solution. The stimulating pulse was applied through the electrode at the proximal end and the action potential was recorded by the distal electrode. A Grass SD9 stimulator was used for electrical stimulation (2 V, 50 .mu.s). The signal was transmitted to a Medelec PA63 preamplifier and thence to a Medelec MS7 electromyograph and AA7T amplifier. The solution, stimulator and amplifier had a common ground. The maximum amplitude of eight averaged CAPs was recorded and photographed with a Polaroid camera. The left nerves (uninjured) were used to measure the reference values of normal nerves and to calibrate the crush forceps.

39 Recording of Visual Evoked Potential (VEP) Response

40 Injured drug-treated rats were examined in 2 weeks after the injury for assessment of their functional recovery. In this set of experiments, the pattern of filed potentials in response to light stimulation was recorded from the primary visual cortex. The potential evoked by the light originates in the retina and is propagated along the surviving axons to reach their final target, the visual cortex. Only those axons that survived the primary and secondary degenerative processes are capable of conducting an action potential. A comparative analysis of the pattern of field potentials in treated and untreated animals will reveal the effect of the treatment on axonal survival.

41 Anesthetized rats (Rumpon, Ketalar) were placed in a small animal sterotaxic instrument. After exposure of the skull, two holes were drilled with a cylindrical drill bit, with the dura kept intact to minimize cortical damage. One hole, drilled above the nasal bone, was used as a reference point. The second hole was in the area OC1 with the coordinates Bregma # 8 mm, lateral # 3 mm. A gold contact pin connected to a screw was used as the electrode, which was screwed into the holes and glued by acrylic cement to the skull. The field potential was evoked by stroboscopic stimulation, with an average of 90 sweeps per minute. The flash-evoked potential was analyzed by the use of the Lab View data acquisition and management system. The field potentials were digitized and stored for off-line analysis.

42 Part C

43 Measurement of Effects of Drug Tests for Neural Protective Properties

44 The first set of experiments involved metabolic measurements. Each drug was injected intraperitoneally at several different concentrations. Each drug was tested in a group of 8 animals, together with 8 controls (injured animals treated with the buffer vehicle). In each case, metabolic measurements were obtained on-line before injury, 0.5 h after injury and every hour for 4-6 h thereafter. The data obtained were analyzed by ANOVA.

45 Measurement of Long Term Effects

46 Physiological Activities

47 CAPS

48 Immediately after injury, the drug to be tested was injected into 10 animals, and 10 control animals were injected with vehicle. Two weeks later the CAPs of each nerve were recorded in vitro, using suction electrodes. The contralateral side was used as an internal control. The results indicated whether the examined drug had any potential effects on the rescue of spared axons and/or slowing of degeneration. Positive results led to efforts at determining the optimal dosage for each promising drug.

49 VEP Response

50 Electrodes were implanted in the cortex of naive SPD rats in two age- and sex-matched groups. Immediately after implantation, the VEP response was recorded from the left side while a light was flashed into the right eye, with the left eye covered. A well-controlled crush injury was then inflicted on the optic nerve and the drug was immediately administered at the previously determined optimal dosage. Control animals were handled in the same way except vehicle was administered rather than drug. The VEP response for each animal was recorded 1 day, 1 week, 2 weeks and 4 weeks after operation.

51 While this invention has been described with respect to various specific examples and embodiments, it is to be understood that the invention is not limited thereby and should only be construed by interpretation of the scope of the appended claims.

CLAIMS:

What is claimed is:

1. A method of protecting the retinal or optic nerve cells in a mammal suffering a noxious action or at risk of experiencing a noxious action on said nerve cells comprising administering to said mammal an amount and dosage regimen of a compound of formula I effective to inhibit or prevent nerve cell injury or death ##STR7## wherein the 2-imidazolin-2-ylamino group is in either the 5- or 6-position of the quinoxaline nucleus; x, y and z are in any of the remaining 5-, 6-, 7- or 8-positions and are selected from hydrogen, halogen, lower alkyl, lower alkoxy or trifluoromethyl; and R is an optional substituent in either the 2- or 3-position of the quinoxaline nucleus and may be hydrogen, lower alkyl or lower alkoxy, or pharmaceutically acceptable salts thereof and mixtures thereof.

2. The method of claim 1 wherein the noxious action is diabetic retinopathy.

3. The method of claim 1 wherein the noxious action is non-glaucoma tous ischemia.

4. The method of claim 1 wherein the noxious action is microangiopathic in nature and is a symptom of the disease chosen from the group consisting of polyarteritis nodosa, giant cell angitis, aortitis syndrome and systemic lupus erythematosus.

5. The method of claim 1 wherein oral administration is used to supply the compound to the mammal systemically.

6. The method of claim 5 wherein the amount of the compound administered is from 5-15 mg/kg.

7. The method of claim 1 wherein intrabulbar injection in the eye is used to supply the compound to the mammal.

8. The method of claim 1 wherein parenteral administration is used to supply the compound to the mammal systemically.

9. The method of claim 1 wherein intramuscular injection is used to supply the compound to the mammal systemically.

10. The method of claim 1 wherein the compound of formula I has the 2-imidazolin-2-ylamino group at the 6-position of the quinoxaline ring, y and z are both hydrogen and located at the 7- and 8-positions and x is at the 5-position of the quinoxaline ring.

11. The method of claim 1 wherein the compound of formula I is ##STR8##

12. The method of claim 1 wherein the compound of formula I is ##STR9##

13. The method of claim 1 wherein the compound of formula I is ##STR10## wherein

x is as defined in claim 1 and the noxious action is diabetic retinopathy.

14. The method of claim 1 wherein the compound of formula I is ##STR11## wherein x is as defined in claim 1 and the noxious action is non-glaucomatous ischemia.

15. The method of claim 1 wherein the compound of formula I is ##STR12## wherein x is as defined in claim 1 and the noxious action is microangiopathic in nature and is chosen from the group consisting of polyarteritis nodosa, giant cell angitis, aortitis syndrome and systemic lupus erythematosus.